

IN THE CLAIMS:

Claim 1 (currently amended) A method for synthesizing a target polynucleotide that is efficiently expressed in a host-vector expression system, comprising the steps of:

(1) conducting a first polymerase chain reaction on a first template with a first primer pair to obtain a first polymerase chain reaction product; wherein which ~~is characterized in that~~ the first template is any template sequence commonly used in the host-vector expression system or a fragment of the target polynucleotide;

(2) conducting multi-cyclic polymerase chain reactions by a primer extension technique to obtain a product comprising the target polynucleotide sequence; ~~wherein the template used in each polymerase chain reaction~~ of the multi-cyclic polymerase chain reactions is conducted on a template that is a product is the product obtained in a the previous polymerase chain reaction; and wherein each polymerase chain reaction of the multi-cyclic polymerase chain reactions is conducted with one or more sets of primer pairs selected from the group consisting of

~~which is characterized in that the primer pairs used in the polymerase chain reactions are designed to be any one of the following three primer pairs:~~

(i) a second set of primer pairs consisting of a forward primer and a reversed primer, the forward primer having two parts:

(a) the part (a1), ~~located~~ located at the ~~5-~~ 5' end region of the forward

primer, comprising a fragment having more than 10 nucleotides and being homologous to the fragment at the 3' end region of the target polynucleotide sequence, and

(b) the part (b1), ~~locating~~ located at the 3' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the sequence of the more than 10 nucleotides from the 5' end region of the template sequence;

and wherein the 3' end of the part (a1) is adjacent to the 5' end of the part (b1); and the reversed primer having, at the 3' end region of the reversed primer, a fragment having more than 5 nucleotides and being capable of annealing to the 3' end region of the template sequence;

(ii) a third set of primer pairs consisting of a forward primer and a reversed primer, the forward primer having at the 3' end region of the forward primer, a fragment having more than 5 nucleotides and being homologous to the 5' end region of the template sequence; and the reversed primer having

(a) ~~the part (a2), locating~~ located at the 5' end region of the reversed primer, comprising a fragment having more than 10 nucleotides and being complement to the 5' end region sequence of the target polynucleotide sequence;

(b) ~~the part (b2), locating~~ located at the 3' end region of the reversed primer, comprising a fragment having more than 10 nucleotides and be

capable of annealing to the sequence of the more than 10 nucleotides from the 3' end region of the template sequence, and wherein the 3' end of the part (a2) is adjacent to the 5' end of the part (b2); and

(iii) a fourth set of primer pairs consisting of a forward primer and a reversed primer, the forward primer having

(a) the part (a3), ~~locating~~ located at the 5' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the fragment at the 3' end region of the target polynucleotide sequence;

(b) the part (b3), ~~locating~~ located at the 3' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the sequence of the more than 10 nucleotides from the 5' end region of the template sequence

and wherein the 3'-end of the part (a3) is adjacent to the 5' end of the part (b3); and the reversed primer having

(c) the part (c3), ~~locating~~ located at the 5' end region of the reversed primer, comprising a fragment having more than 10 nucleotides and being complement to the 5' end region of the target polynucleotide sequence;

(d) the part (d3), ~~locating~~ located at the 3' end region of the reversed primer, comprising a fragment having more than 10 nucleotides and annealing to the sequence of the more than 10 nucleotides from the 3'

3' end region of the template sequence; and wherein the ~~3~~ 3' end of the part (c3) is adjacent to the ~~5~~ 5' end of the part (d3); and wherein all of the fragments of the target polynucleotide sequence used in the polymerase chain reactions in sequence constitute the target polynucleotide sequence; and

(3) ~~obtaining-recovering~~ the polynucleotide product comprising the target polynucleotide sequence from the final product of the multi-cyclic polymerase chain reactions.

Claim 2 (currently amended) The method according to Claim 1 wherein the first template comprises a sequence not found in the target polynucleotide sequence, and wherein the method further comprises ~~further comprising~~ a step of removing the nucleotide sequence of the first template from the final product in the step (3) so as to obtain a product consisting of the target polynucleotide sequence ~~if the first template is irrelevant to the target polynucleotide sequence.~~

Claim 3 (currently amended) The method according to Claim 2, wherein the first template is designed to have restriction enzyme recognition sites at ~~the~~ both ends.

Claim 4 (currently amended) The method according to Claim 1, wherein the

fragment having more than 10 nucleotides used in each step ~~is~~ has more than 15 nucleotides.

Claim 5 (currently amended) The method according to Claim 1, ~~wherein~~ further comprising determining if the target polynucleotide sequence is heterogeneous to ~~a~~ the host to be used in expressing the protein encoding the target polynucleotide, and adjusting a sequence of the one or more sets of primer pairs to change a codon ~~some codons of the target polynucleotide are changed to the codons to a codon~~ which ~~have~~ has a high expression efficiency in translating ~~the same~~ a corresponding amino acid in ~~the host~~ a cell of the host.

Claim 6 (currently amended) The method according to Claim ~~5~~ 4, wherein the host is an enteric bacterium.

Claim 7 (previously presented) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 1;
- (2) transforming or transfecting the target polynucleotide to the host; and

(3) expressing the target heterogeneous protein in the transformed or transfected host.

Claim 8 (original) The method according to Claim 7, wherein the host is an enteric bacterium.

Claim 9 (currently amended) The method according to Claim 8, which further comprises, adjusting a sequence of the one or more sets of primer pairs to change in the fragments of the target polynucleotide used for expressing the target heterogeneous polypeptide, by changing the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine changed to GGT or GGC; the codon CCC encoding proline to CCG, CCA or CCT; the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine to GGT or GGC; or the codon CCC encoding proline to CCG, CCA or CCT.

Claim 10 (currently amended) The method according to Claim 1, wherein the target polynucleotide encodes a mutated protein which has multiple mutation sites ~~comparing to the~~ compared to a wild-type form thereof.

Claim 11 (currently amended) The method according to Claim 1, wherein the first polymerase chain reaction in the step (1) ~~further~~ is conducted ~~by~~ with a helper primer which is homologous to one primer of the first primer pair and identical to a fragment of one strand of the target polynucleotide.

Claim 12 (previously presented) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 2;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.

Claim 13 (previously presented) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 3;
- (2) transforming or transfecting the target polynucleotide to the host; and

(3) expressing the target heterogeneous protein in the transformed or transfected host.

Claim 14 (previously presented) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 4;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.

Claim 15 (previously presented) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 5;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.



Claim 16 (new). The method according to claim 1, wherein the first polymerase chain reaction is conducted with primers that consist of the first primer pair.

Claim 17 (new). The method according to claim 11, wherein the first polymerase chain reaction is conducted with primers that consist of the first primer pair and the helper primer.

Claim 18 (new). The method according to claim 1, wherein the primers of the first primer pair and the primers of the multi-cyclic polymerase chain reactions comprise no more than 36 nucleotides.

Claim 19 (new). The method according to claim 1, wherein each of the multi-cyclic polymerase chain reactions is conducted only on a template that consists of a product that has been extended with the addition of nucleotides in a previous polymerase chain reaction whereby the product of each succeeding polymerase chain reaction is longer than the product of each previous polymerase chain reaction.